

Figure 1

09687959-101300

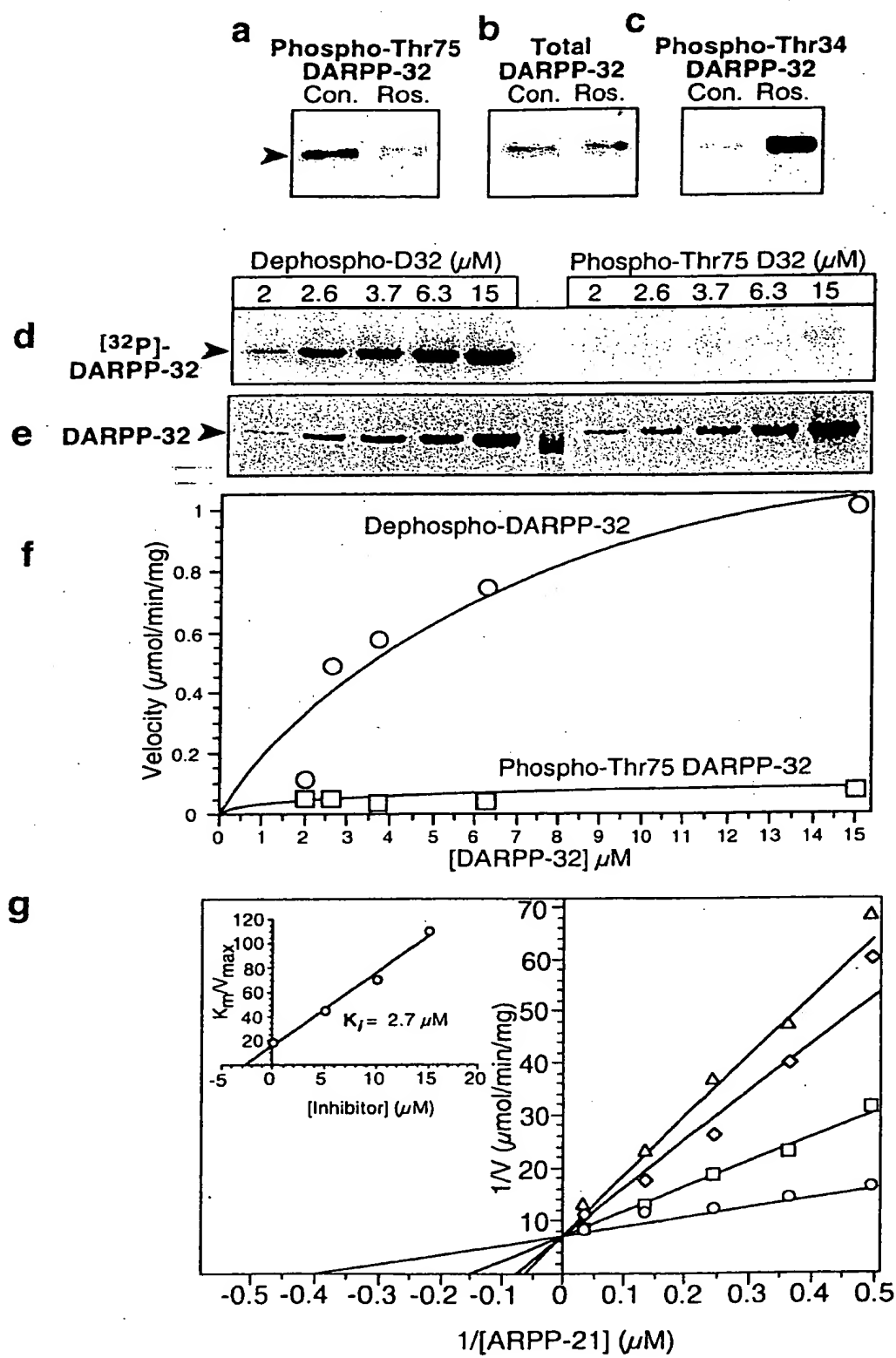
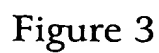


Figure 2

00687959-101300



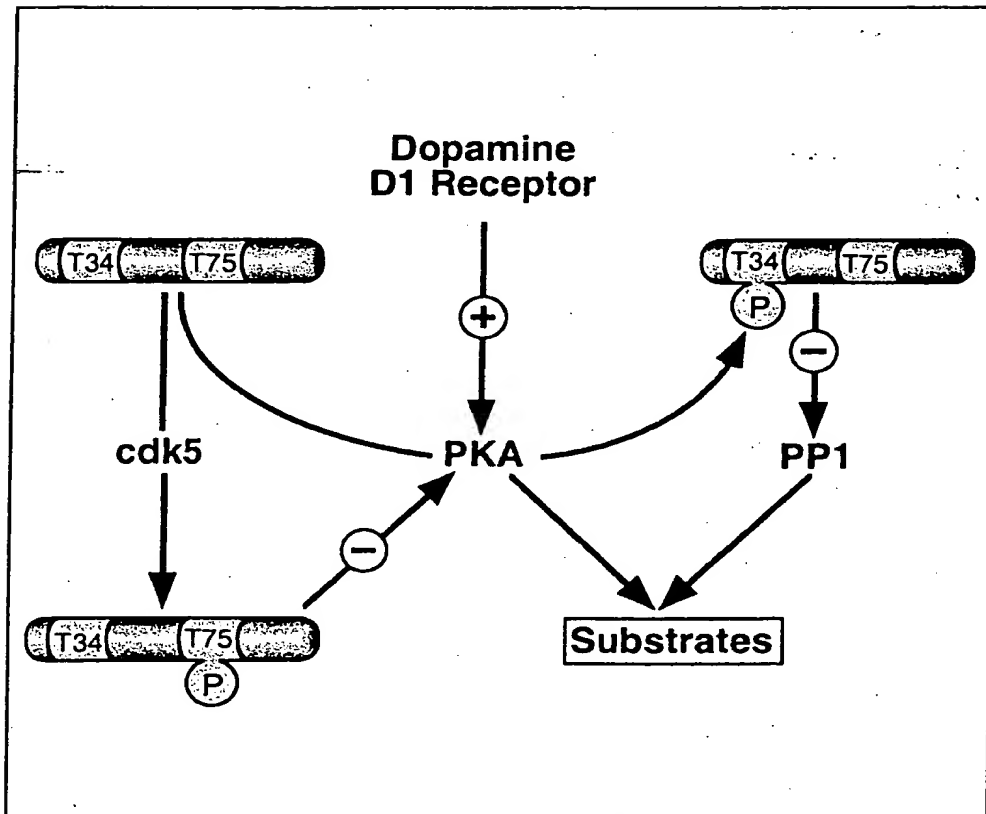


Figure 4A

0056707" 65648960

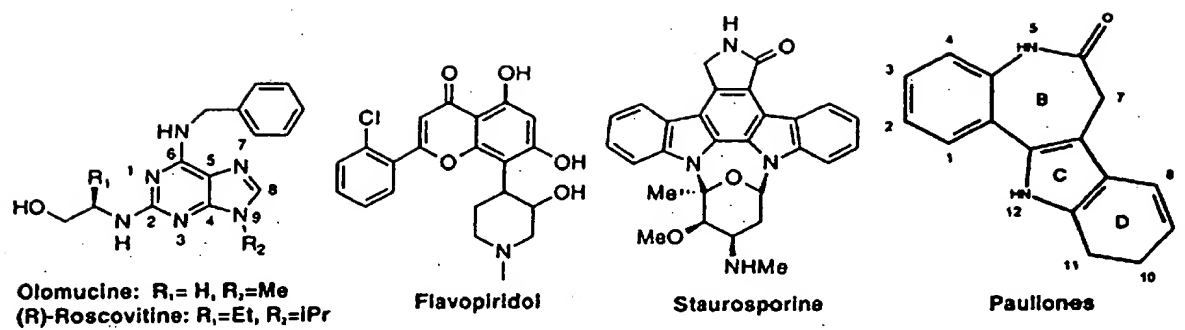
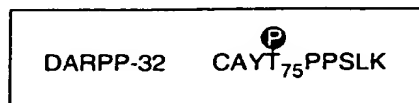


Figure 5

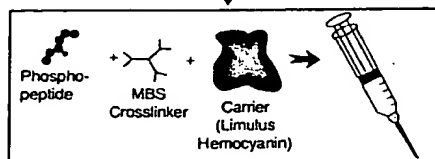
Production of Phosphorylation State-Specific Antibody

A



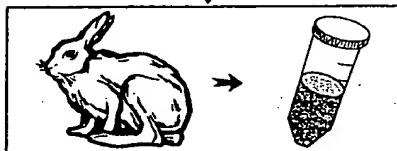
Following determination of the phosphorylated residue, a short synthetic phospho-peptide corresponding to the region surrounding the site is prepared.

B



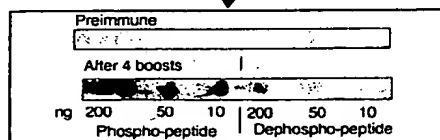
Phosphopeptides are conjugated to carrier protein and prepared for inoculation.

C



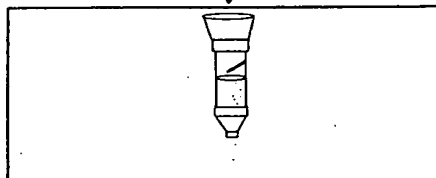
Inoculation, boosting, and bleeding of rabbits.

D



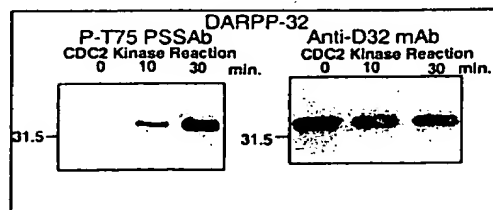
Primary screening of anti-sera by immuno-dot-blotting.

E



Purification of phospho-specific Ab using protein A and phospho/dephosphopeptide affinity chromatography.

F



Confirming specificity by immunoblotting

Steps involved in the generation of phosphorylation state-specific antibodies (PSSAb) are illustrated schematically. Typical results of phospho-peptide dot-blot screening of serum are shown. The bottom panel shows immunoblot results confirming the selectivity of the antibody for phosphorylated protein.

Figure 6

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Human:	1	MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAML	34	PRLSEHSSPEEE	50
Bovine:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAMLFRLSEHSSPEEE			
Rat:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPALLFRVSEHSSPEEE			
Mouse:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAMLFVRVSEHSSPEEE			
Human:		ASPHQRASGEGHHLKSKRPNPCAYTPPSLKAVQ	75	RIAESHLOSISN	100
Bovine:		ASPHQRASGEGHHLKSKRSNP		CAYTPPSLKAVQ	
Rat:		SSPHQRTSGEGHHPKSKRPNPCAYTPPSLKAVQ		RIAESHLOTISN	
Mouse:		ASPHQRTSGEGHHPKSKRPNPCAYTPPSLKAVQ		-----HLQ	
Human:	102	ASEEDELGELRELGYPRE	137	EDDEEEEDDEEEEDSQA	150
Bovine:		ASEEDELGELRELGYPRE		EDDEEEEDDEEEEDSQA	
Rat:		ASEEDELGELRELGYPRE		EDDEEEEDDEEEEDSQA	
Mouse:		ASEEDELGELRELGYPRE		EDDEEEEDDEEEEDSQA	
Human:		TTRGLGLEGPWQRPPPLDES		ERDGGSEDQVEDPAL-SEP	200
Bovine:		TTYGQGLEGPWERPPPLDGP		QRDGGSEDQVEDPALN-EP	
Rat:		LTSGQGLEGPWERPPPLDEP		QRDGNSEDQGEGRATQSEP	
Mouse:		PTCGRGLEGPWERPPPLDEP		QRDGNSEDQVEGRATLSEP	
Human:	P-GR	199			
Bovine:	P-GT	198			
Rat:	ESGT	201			
Mouse:		194			

Figure 7

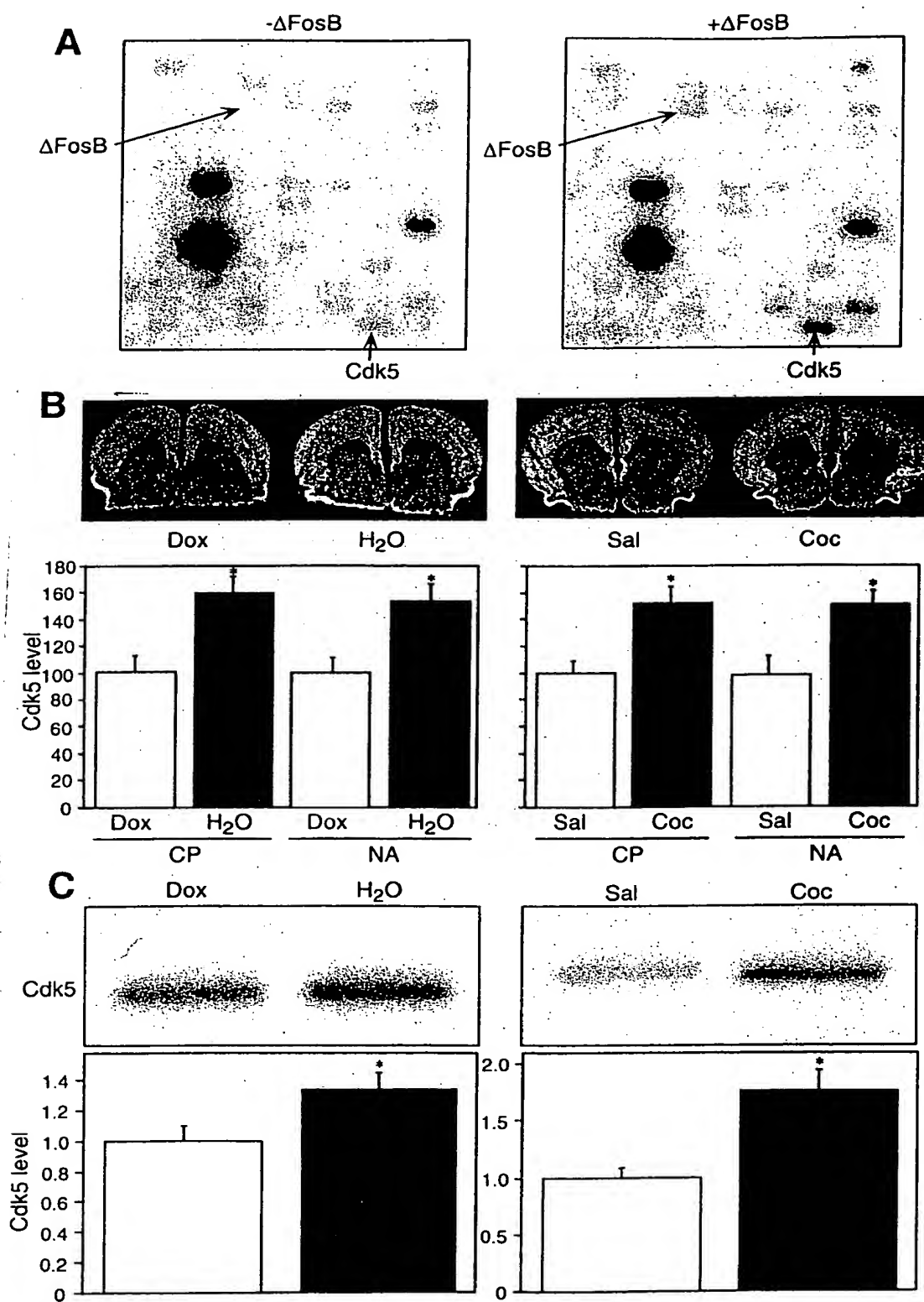


Figure 8

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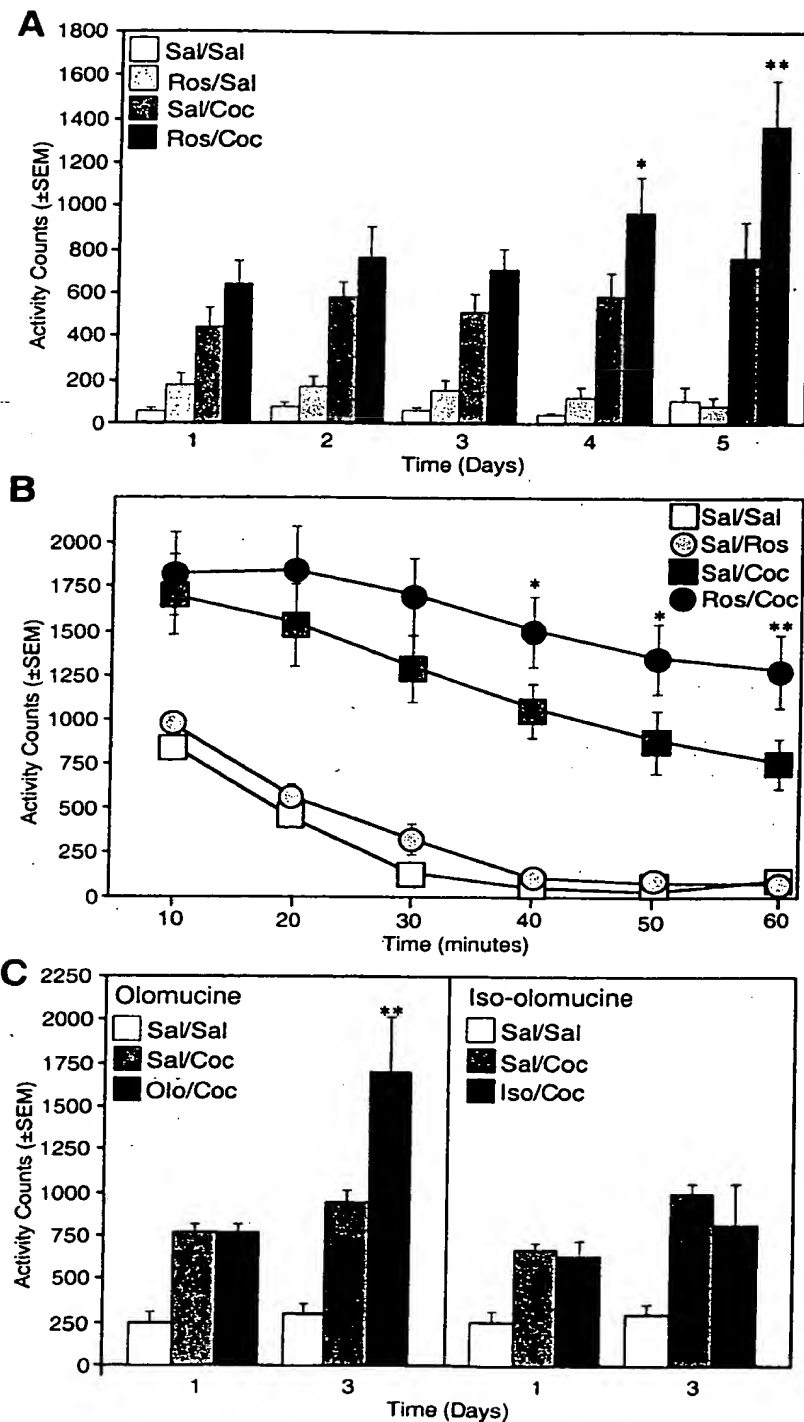


Figure 9

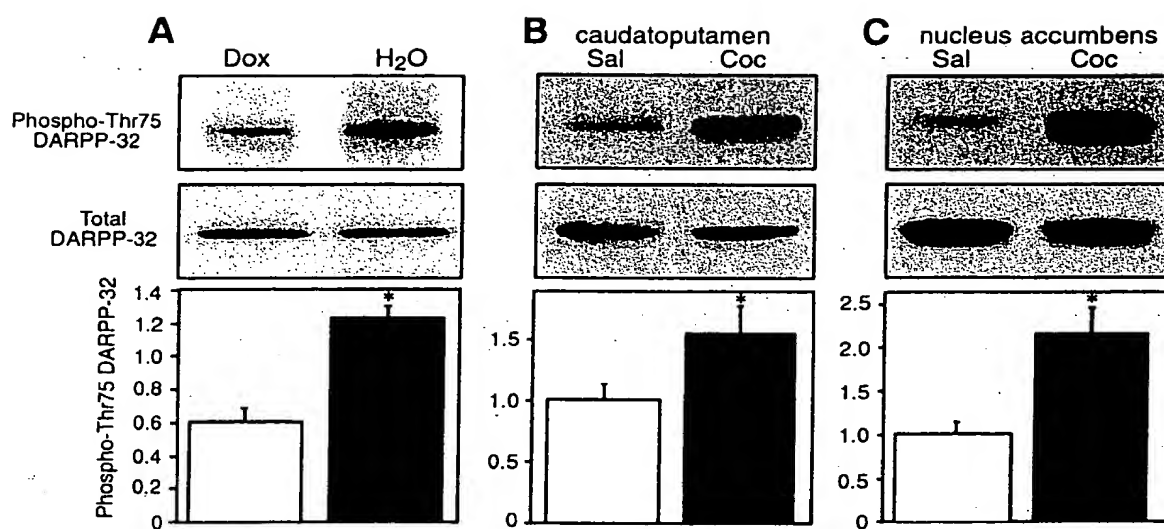


Figure 10

